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***In Vitro* Fermentation Characteristics, Feeding Behavior, and Preference of Growing Holstein Dairy Heifers to a Modified Lignin Product**

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Five feeds were prepared to meet the nutrient requirements of growing Holstein heifers using two different binders in a pelleted form: a negative control that contained neither molasses nor the new product (treatment 1), a positive control that contained molasses (2% DMB, treatment 2), and three pelleted feeds that contained a new binder in varying concentrations – low (1.0% DMB, treatment 3), medium (2.5% DMB, treatment 4), high (4.0% DMB, treatment 5) – plus molasses (2% DMB). Feeding behavior was recorded via placing heifers (n=8 per treatment) into individual pens (3 × 5 m) with one pelleted feed offered at a time over 60 min period to evaluate feed acceptance. Feeding preference was determined by offering two feed bunks at a time: one containing a reference diet (negative control) and the other containing one of the test diets (one of five treatments). Intake was measured and preference was calculated using the following formula: Preference % = (Test diet) / (Test diet + Reference diet) × 100%. The five feeds were then placed in the rumen fluid for 3, 6, 9, 12, 18, 24, and 48 hours *in vitro*. After incubation, the dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) degradation were determined. Data were analyzed using the Proc Glimmix of SAS (v. 9.4) with animals as the random effect. Significance was declared at $P \leq 0.05$ and trends at $P < 0.1$. Results showed that the animals accepted the pelleted feed containing the high inclusion modified lignin product more than the other feeds ($P < 0.05$). Heifers spent more time ruminating ($P < 0.05$) and eating ($P < 0.05$) on negative control compared with that of other feeds except for the low inclusion. Results also showed that there was a significant effect of treatment on preference (68.0, 69.2, 68.4, 77.8, and 79.0% ± 3%, treatment 1 to 5 respectively, $P < 0.05$) among treatments with treatments 4 and 5 being more preferred over control feeds. There was a significant effect of treatment on *in vitro* DM degradation (32.7, 32.9, 33.9, 35.8, and 36.1 ± 0.53%, treatment 1 to 5, respectively; $P < 0.05$) among treatments. The degree of DM degradation for treatment 4 and 5 was greater than treatment 1, 2 and 3. Significant effect of treatment on *in vitro* OM degradation was observed (29.8, 32.5, 35.2, 36.1, and 38.9 ± 0.77%, treatment 1 to 5, respectively; $P < 0.05$). *In vitro* OM degradation of treatment 5 showed the greatest value among treatments. *In vitro* NDF degradation at 48 hours did not however differ significantly among treatments (23.3, 11.78, 15.6, 17.47, and 23.38 ± 3.8%, treatment 1 to 5, respectively; $P = 0.14$). There was a trend of *in vitro* degradation of ADF at 48 hours over five treatments ($P < 0.1$). Research is needed to evaluate nutritional value of modified lignin products in animal production settings.

Keywords: feeding behavior, feed binder, pelleted feed, growing heifer

Intake and *in Vito* Fermentation Characteristics of Pelleted Feeds Containing Different Binders in Growing Primiparous Holstein Cows Diets

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Five pelleted feeds were prepared to meet the nutrient requirements of growing dairy Holstein heifers. They included a negative control containing neither molasses nor the new product (CTRL-N; negative control), a positive control that contained Ameri-Bond 2X at 2.1% dry matter basis (DMB, CTRL-P; positive control). Three pelleted feeds that contained a new binder in varying concentrations were low (1.6% DMB, DMB-L), medium (3.2% DMB, DMB-M), high (4.8% DMB, DMB-H) inclusion rates. Ten Holstein heifers were placed in individual stalls, and given one feed for one hour to determine the acceptance and intake of each feed. The five treatments were then placed in the rumen fluid for different lengths of time – 0, 4, 8, 12, 18, 24, 48 and 72 hours. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash were analyzed after incubation. Data were analyzed using the PROC GLIMMIX of SAS (v. 9.4). Significance was declared at $P \leq 0.05$. Preliminary results showed a significant effect of treatment on DM intake (3.72 ± 0.28 , 4.41 ± 0.29 , 5.11 ± 0.28 , 3.62 ± 0.28 , and 4.00 ± 0.28 kg, CTRL-N to DMB-H, respectively, $P = 0.001$). Intake of DMB-L (low inclusion) was the highest among all treatments while intake of CTRL-P (positive control) did not differ from that for CTRL-N (negative control), DMB-M (medium) or DMB-H (high). Preliminary results showed that there was a significant effect on overall DM *in vitro* degradation (32.0 , 34.4 , 35.2 , 34.0 , and $33.4\% \pm 0.52\%$, CTRL-N to DMB-H, respectively, $P = 0.0004$) among treatments. The degree of DM degradation for CTRL-P (positive control), DMB-L (low), and DMB-M (medium) were greater than that for CTRL-N (negative control) and DMB-H (high). Overtime organic matter *in vitro* degradation of CTRL-P- through DMB-H were all greater than CTRL-N. Overtime *in vitro* NDF degradation differed among treatments ($P = 0.002$). While NDF degradation did not differ at 0, 24 or 72 h, they differed at 48 h with CTRL-N, DMB-L, and DMB-H not differing from each other but greater than CTRL-P and DMB-M. There was a significant difference in ADF *in vitro* degradation by treatment ($P = 0.005$). Overall *in vitro* ADF degradation of CTRL-N was greater than that of other treatments while difference at 48 h and 72 h showed that DMB-L and DMB-H did not differ from CTRL-N (negative control). Research is continuing to determine the preference of pelleted feeds containing various feed binders.

Keywords: feeding behavior, feed binder, pelleted feed, growing heifer

Effect of feeding supplemental zeolite on measures of nitrogen utilization in backgrounding cattle

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Although it could potentially reduce the loss of ammonia-N from the rumen into blood as a result of its high ion exchange affinity for cations including ammonium ions, the impact of feeding zeolite on nitrogen (N) excretion and reactive N emissions remain to be determined in cattle. Therefore, the objective of this study was to evaluate the effects of feeding supplemental zeolite with a particle size of either 30 microns or 400 microns (US 40 mesh) on ruminal ammonia-N and plasma urea-N concentrations, and N excretion in backgrounding cattle. A total of 6 cannulated beef heifers were used in a replicated 3 × 3 Latin square design with 21-d periods. The dietary treatments were: 1) a typical forage-based backgrounding TMR with no supplement (CON), 2) CON + 30-micron zeolite (ZEO-30), and 3) CON + 400-micron zeolite (ZEO-400). The supplementation rate was 2.5% of diet DM, with the zeolite top-dressed during morning feeding (1100 h). Ruminal fluid (d 19 at 2 h post-feeding), blood (d 21 at 3 h post-feeding), and spot urine samples (d 19 at 1400 and 1900; d 20 at 0300, 0700, 1500, and 2300 h; d 21 at 0500 and 1100 h) were collected and analyzed for metabolites. All data were analyzed using the MIXED procedure of SAS. Dry matter intake was lower ($P = 0.047$) for heifers fed the ZEO-30 diet compared to the CON and ZEO-400 diets (16.3, 15.1, and 15.6 kg/d for CON, ZEO-30, and ZEO-400, respectively). However, there was no diet effect ($P \geq 0.19$) on ruminal ammonia-N concentration (14.6, 14.0, and 12.8 mg/dL for the CON, ZEO-30, and ZEO-400 diets, respectively). Similarly, plasma urea-N concentration did not differ ($P = 0.91$) across diets. Urine output (12.5, 11.2, and 11.1 L/d for the CON, ZEO-30, and ZEO-400 diets, respectively) and urine urea-N output (104, 96, and 93 g/d for the CON, ZEO-30, and ZEO-400 diets, respectively) also did not differ ($P \geq 0.39$) across diets. In summary, although feeding ZEO-30 to beef heifers resulted in an undesirable decrease in DMI, both ZEO-30 and ZEO-400 did not result in changes in measures of N utilization.

Keywords: ruminal nitrogen metabolism, nitrogen excretion, zeolite

***In vitro* rumen fermentation characteristics of high-grade crystalline vs. low-grade liquid betaine products**

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Betaine, a co-product of sugar-beet processing, can be used to feed cattle. Because high-grade betaine (> 90% pure; dry matter (DM) basis) is expensive, feed-grade products with lower betaine concentration are typically used in cattle rations. However, there is limited information on the impact of feeding the feed-grade betaine products on rumen fermentation characteristics. Therefore, our objective was to compare *in vitro* rumen fermentation characteristics of a high-grade betaine (97% purity) to a feed-grade betaine product (32% purity). The ANKOM gas production system was used (ANKOM Technologies, Macedon, New York, USA) to determine the *in vitro* fermentation characteristics of both products at the same inclusion level. Three dietary treatments were used: control (CON) with no betaine added, high-grade crystalline betaine (CRYS), and feed-grade liquid betaine (LB50) at 0.50% of diet DM. The study was a completely randomized design and each treatment was added to 2 ANKOM modules, which contained 1.5 grams of total mixed ration, 15 mL rumen fluid, and 45 mL McDougall's buffer. Two ANKOM modules were also used as blank/run. A total of 3 runs were conducted, each run lasted for 24-hours. Data were analyzed using the mixed procedure of SAS. Crystalline betaine had a greater crude protein content compared to the liquid betaine (72.8 vs 56.7 % DM). Total volatile fatty acid production tended to be greater in LB50 vs CRYS (140.23 vs. 109.14 mM respectively, $P = 0.09$) while no differences ($P > 0.1$) were detected in the molar proportions of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate, which averaged 49.15 ± 0.81 , 29.67 ± 0.69 , 13.78 ± 0.51 , 1.27 ± 0.04 , 3.50 ± 0.12 , 2.06 ± 0.09 , and 0.58 ± 0.07 % respectively. Final pH did not differ ($P = 0.27$) among treatments and averaged 6.20 ± 0.02 . Similarly, *in vitro* true DM digestibility and methane production did not differ ($P \geq 0.15$) among treatments. In summary, the lack of differences in *in vitro* fermentation characteristics between an expensive high-grade and a lower-grade betaine product suggests a similar feeding value when fed at the same dietary inclusion rate.

Keywords: *In-vitro* fermentation, VFA, betaine

***In vitro* fermentation characteristics of Cheatgrass (*Bromus tectorum* L.) and Medusahead (*Taeniatherum caputmedusae* L.) harvested on Idaho rangeland in different seasons**

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Grazing could be used to control invasive grass species on rangeland. However, forage digestibility, which can change as plants mature, influences the amount that cattle can graze. Therefore, the objective of this study was to determine *in vitro* fermentation characteristics of Cheatgrass (*Bromus tectorum* L.) and Medusahead (*Taeniatherum caputmedusae* L.) harvested on Idaho rangeland in summer (June 2018), fall (September 2018) and winter (January 2019). Forage samples were collected from the Reynold Creek watershed (Owyhee county, ID). A batch culture system was then used to evaluate volatile fatty acid (VFA) production (6 h), and 24 h *in vitro* dry matter (IVDMD) and NDF digestibility (IVNDFD). All data was analyzed using the MIXED procedure of SAS. Although the crude protein (CP) content was greater for Cheatgrass than Medusahead in summer (14.1 vs. 10.6%; DM basis), it did not differ in fall and winter. However, for both grasses, there was a dramatic decrease in the CP content in fall and winter (average of 3.9% for both seasons). The TDN content was also greater for Cheatgrass than Medusahead in summer (66% vs. 55%), with the content decreasing dramatically in both grasses in fall and winter. However, the NDF content of both grasses increased with advancing maturity. There was no season or grass species effect ($P \geq 0.24$) on total VFA concentration and the molar proportion of acetate. However, there was a season \times grass species interaction ($P = 0.04$) for the molar proportion of propionate; it was greater for Cheatgrass than Medusahead in summer, but not in winter and fall. There was a season \times grass species interaction ($P < 0.01$) for 24 h IVDMD and IVNDFD; both IVDMD and IVNDFD were greater ($P < 0.01$) for Cheatgrass than Medusahead when harvested in summer (62.9% vs. 51.3% and 49.7% vs. 44.9%, respectively), with no differences across grass species in winter and fall. However, IVDMD and IVNDFD for both grasses decreased by 45 to 50% and 47 to 59%, respectively, as the season advanced from summer to fall and winter. In summary, forage quality was greater for Cheatgrass than Medusahead in summer; however, there was a dramatic and comparable decrease in DM and fiber digestibility for both grasses beyond summer, which possibly could compromise forage intake, thus, limiting the effectiveness of grazing as a tool to control their spread on rangeland.

Keywords: *in vitro* fermentation, invasive grass species, Idaho rangeland

Impacts of heifer post-weaning residual feed intake classification on reproductive and performance measurements of first, second and third parity Black Angus females

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The objectives of this study were to evaluate heifer post-weaning residual feed intake (RFI) classification on reproductive and performance measurements of first, second and third parity Black Angus females. We analyzed the annual as well as cumulative production of 347 Angus females from birth through weaning of their 3rd calf. Heifer post-weaning RFI was calculated as the actual dry matter intake minus the predicted dry matter intake based on the average daily gain of the contemporary group on an annual basis and ranged from -1.99 to +1.71 kg as fed d⁻¹ (SD = 0.55). Heifers were categorized as either low (< -0.50 SD from mean), or average (+/- 0.50 SD from mean) or high (> +0.50 SD from the mean) within year. Julian birth day of heifers was influenced by RFI classification ($P < 0.01$) and displayed both linear ($P = 0.05$) and quadratic ($P = 0.02$) effects with high RFI calves being born earlier in the calving season than average or low RFI calves (71.2 vs 75.3 days). Cow birth weight, weaning weight, as well as yearling weight and body condition were not influenced by RFI classification ($P > .05$). Parity number differed related to weight with third parity pregnancy weights being heavier than second and first, and second parity pregnancy weights being heavier than first but lighter than third pregnancy ($P < 0.01$) with no RFI or RFI*parity interaction ($P > 0.05$). Calf birth weights differed by RFI classification ($P < 0.03$) and parity ($P = 0.01$) with second and third parity calves having heavier birth weights than first parity calves, however, no RFI*parity interaction was observed ($P > 0.05$). Calf 205 day weaning weights and weaning weight ratio was influenced by parity ($P < 0.05$) with increasing weaning weights and decreasing weaning weight ratio for the first calf to the 3rd calf. In contrasts, RFI classification had no effect on weaning weights or weaning weight ratios ($P > 0.05$). Cow conception probability differed by year of pregnancy ($P < 0.05$) and a RFI*pregnancy interaction ($P = 0.02$) was observed with younger low RFI cows tending to have lower conception rates. Cow AI conception probability differed by year of pregnancy ($P < 0.01$) but not RFI classification ($P > 0.05$). In summary, heifer post-weaning RFI classification had minimal effects on beef cattle production and reproductive efficiency.

Key words: beef cattle, heifer, parity, production, reproduction, residual feed intake (RFI)

Effect of feeding ensiled or dried grape pomace on measures of nitrogen utilization in backgrounding cattle

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Because of its content of polyphenolic compounds, feeding grape pomace could alter ruminal and whole-body nitrogen (N) utilization in cattle in a manner that reduces N wastage. However, the preservation method used for grape pomace, whose shelf-life is limited when fresh, could potentially cause changes in the bioactivity of the polyphenolic compounds. Therefore, the objective of this study was to evaluate the effects of feeding either ensiled or sun-dried grape pomace on ruminal ammonia-N and plasma urea-N concentrations, and N excretion in backgrounding cattle. A total of 6 cannulated beef heifers were used in a replicated 3×3 Latin square design with 21-d periods. The dietary treatments were (DM basis): 1) a typical backgrounding diet (CON), 2) CON + 15% ensiled grape pomace (ENS), and 3) CON + 15% sun-dried grape pomace (DRY). The grape pomace partially replaced triticale silage in the diet. Ruminal fluid (d 19 at 3 h post-feeding), blood (d 21 at 3 h post-feeding), and spot urine samples (d 19 at 0900, 1500, and 2100; d 20 at 0300, 1200, and 1800 h; d 21 at 0000 and 0600 h) were collected and analyzed for metabolites. Ruminal pH was also measured at 3 and 4 h post-feeding on d 19. All data were analyzed using the MIXED procedure of SAS. There was no diet effect ($P \geq 0.37$) on dry matter intake (DMI) and ruminal pH at 3 h post-feeding. However, ruminal pH at 4 h post-feeding was lower for heifers fed the DRY compared to the CON and ENS diets (6.19, 6.07, and 6.30 for the CON, ENS, and DRY diets, respectively). There was no diet effect ($P = 0.45$) on ruminal NH_3 -N concentration. Similarly, plasma urea-N concentration ($P = 0.97$) and urine output ($P = 0.30$) did not differ across diets. However, urine urea-N output was lower ($P < 0.01$) in heifers fed diets containing grape pomace than the CON diet (84.9, 62.1, and 67.1 g/d for the CON, ENS, and DRY diets, respectively L/d). In summary, although feeding either ensiled or sun-dried grape pomace had no effect on DMI, and rumen ammonia-N and plasma urea-N concentrations, it resulted in a decrease in urine urea-N excretion, which could be beneficial from an environmental standpoint.

Keywords: grape pomace, preservation method, nitrogen utilization

Impact of a commercial direct-fed microbial on cow performance during the periparturient transition

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The transition period is a metabolically demanding time for dairy animals because of the increased nutrient requirements for milk yield. The objective of this study was to investigate the effect of feeding a commercial direct-fed microbial in multiparous and primiparous dairy cows on productive measures during the transition period. Primiparous (n=33) and multiparous (n=35) cows were fed a close-up TMR before calving and a lactation TMR postpartum. Three weeks before expected calving, all animals were blocked to balance parity and body weight, then randomly assigned to either control group (CTRL; n=34) or a direct-fed microbial (DFM; n=34). The DFM animals received a top-dressed DFM (ProTernative) fed daily at 12.5 g per head. Feed intake was measured by weighing the amount of feed given in one day and subtracting what was left from the total both pre- and post-calving. All animals were weighed weekly for the duration of the study. Blood samples were collected weekly. These samples were analyzed for glucose concentration, and non-esterified fatty acid concentration (NEFA). Colostrum samples were collected at calving and will be analyzed for IgG, IgA, and IgM content. Somatic cell count was also measured in the colostrum samples. Milk samples were collected once per week postpartum, and all of the samples were analyzed for protein percentage, fat percentage, lactose percentage, urea nitrogen (MUN), and somatic cell count (SCC). All results were analyzed using PROC MIXED in SAS with significance defined as $P \leq 0.05$. All covariate models were selected based on the lowest AIC value. Results showed that the interaction of treatment, parity, and time affected DMI where the DFM multiparous cows consumed more feed week 2 postpartum ($P < 0.001$) while DFM primiparous cows consumed more feed weeks 2 and 3 postpartum. Whereas DFM animals were heavier over the experimental period ($P = 0.06$), there was not a significant difference in BW by treatment, parity, or time interaction ($P = 0.11$). The supplementation of the DFM had a significant effect on milk yield as the DFM animals produced more milk overall ($P = 0.02$). There was a significant interaction of treatment, parity, and time on milk protein percentage as the multiparous DFM animals had a greater percentage of protein at week 2 ($P < 0.01$). There was also a significant interaction of treatment, parity, and time on MUN as the DFM primiparous cows had a greater MUN at week 3 ($P = 0.05$). There was not however a significant difference in milk fat percentage ($P = 0.16$), milk lactose ($P = 0.30$), somatic cell count ($P = 0.44$), plasma glucose ($P = 0.16$), or serum NEFA ($P = 0.27$) by main effects or their interactions. Supplementation of a direct-fed microbial improved DMI, milk production, milk protein content, and increased MUN. Gross feed efficiency (energy-corrected milk/DMI) in week 1 postpartum tended to improve by feeding DFM ($P = 0.06$). Further research is needed to better understand the mechanisms involved in enhancing milk yield by DFM.

Key Words: direct-fed microbial, periparturient, dry matter intake

Relationship between nutrient metabolism during the periparturient period and health measures in a Pacific Northwest dairy herd

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During the periparturient period, dairy cows mobilize stored nutrients to support fetal development and milk production. In addition, this period is characterized by increasing risk of metabolic disorders and infectious diseases. Nutrients such as lipid-soluble vitamins affect immune responses. These vitamins may modulate immune responses toward a more anti-inflammatory status and provide protection against invading pathogens. The objective of the present study was to determine the relationship between dairy cows' serum lipid-soluble vitamins and health status during the periparturient period. Blood samples were obtained from a total of 645 periparturient cows on d-21, d-7, d1, d7 and d14 relative to calving. Sera were collected and analyzed for lipid-soluble vitamins (α -tocopherol, β -carotene, and retinol) via HPLC. Health records of the cows were collected and categorized based on the occurrence of periparturient diseases such as lameness, mastitis, and milk fever. The data were analyzed using the Proc Mixed in SAS with significance declared at $P \leq 0.05$. Results showed that there was a significant interaction between the time relative to parturition and seasons on serum α -tocopherol, β -carotene, and retinol concentration regardless of health status ($P < 0.001$). In addition, there was a significant interaction between the time relative to parturition and seasons on serum α -tocopherol, β -carotene, and retinol for cows with lameness ($P < 0.001$). Furthermore, serum α -tocopherol was affected by time, seasons, and mastitis interaction ($P = 0.001$). Cows with mastitis had significantly greater serum retinol concentration compared with that of healthy cows during postpartum ($P = 0.03$). Beta-carotene showed interaction between the time relative to parturition and seasons in cows with mastitis ($P < 0.001$) but not in cows without mastitis. In summary, the metabolic disorders may affect the lipid soluble vitamins status of periparturient cows and may be associated with cows' other health issues. Further research is taking place to determine theses relationship in calves born from the dams with diseases and disorders.

Key words: lipid-soluble vitamin, health status, dairy cow

Effects of Serum Protein Concentrations on Selected Health Measures within the First 90 Days of Life in Holstein Dairy Calf

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Immune response of neonate calf is functional, but naïve and immature; colostrum is a solution to this problem. We hypothesized that increase incidence in morbidity and mortality would be associated with low serum total protein concentration. An objective of this study was to determine the effect of passive transfer status on morbidity and mortality in neonatal Holstein dairy calves (n=1,558). Calves were purchased from several dairy farms in the western United States and placed in a calf ranch as one day old. Calves were assigned an individual electronic identification and entered into Feedlot Health Management Services proprietary software system, iFHMS (Feedlot Health Management Services, Preston, ID). Cause-specific morbidity and mortality was recorded on an individual calf basis daily from entry to exiting or death at the calf ranch. A 5 mL tube of whole blood was collected from each animal at 48 ± 6 h post-arrival. Health events were recorded on an individual calf basis. Whole blood was centrifuged at 2000g for 10 minutes and serum was stored at -22°C until analyzed. Serum total protein (TP) was measured using a digital refractometer as described by (Weaver et al., 2000). Calves were categorized based on proposed USDA serum TP guidelines into poor ($\text{TP} < 5.1$ g/dL), fair ($5.1 < \text{TP} \leq 5.7$ g/dL), good ($5.8 \leq \text{TP} \leq 6.1$ g/dL) and excellent ($\text{TP} > 6.1$ g/dL). Data were analyzed using logistic regression models with significance declared at $P \leq 0.05$ and trend at $P < 0.10$. Results showed that there was a significant difference between poor and excellent in the total respiratory disease treatments as well as the total gastrointestinal disease treatments ($P < 0.001$ for both). In addition, there were differences in the ear disease treatments in relation to serum total protein status (poor vs excellent and fair vs excellent; $P < 0.01$). Higher morbidity was demonstrated in calves with lower serum TP values measured within the first few weeks of life, suggesting that other factors affecting immunity and overall health, such as lipid soluble vitamins, may be involved, which warrants further investigation.

Key words: passive transfer, serum total protein, calf health, lipid soluble vitamins